

Physiological flexibility in the Andean lizard *Liolaemus bellii*: seasonal changes in energy acquisition, storage and expenditure

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Abstract According to the “barrel model”, an organism may be represented by a container, with input energy constraints (foraging, digestion, and absorption) symbolized by funnels connected in tandem, and energy outputs (maintenance, growth, and reproduction) symbolized by a series of spouts arranged in parallel. Animals can respond to changes in environmental conditions, through adjustments in the size of the funnels, the fluid stored inside the barrel, or the output flow through the spouts. In the present study, we investigate the interplay among these processes through the analysis of seasonal changes in organ size and metabolic rate in a lizard species (*Liolaemus bellii*) that inhabits extremely seasonal environments in the Andes range. We found that digestive organ size showed the greatest values during spring and summer, that is, during the foraging seasons. Energy reserves were larger during summer and autumn, and then decreased through winter and spring, which was correlated with overwintering maintenance and reproductive costs. Standard metabolic rate was greater during the high-activity seasons (spring and summer), but this increase was only noticeable at higher environmental temperatures. The ability of many lizard species to reduce their maintenance cost during the cold months of the year, beyond what is expected from temperature decrease, is

probably related to their success in coping with highly fluctuating environments. Here, we demonstrate that this ability is correlated with high physiological flexibility, which allows animals to adjust energy acquisition, storing and expenditure processes according to current environmental conditions.

Keywords Annual cycles · Fat bodies · Gut · Metabolism · Phenotypic plasticity

Abbreviations

BCI	Body condition index
m_b	Body mass
SMR	Standard metabolic rate
SVL	Snout to vent length
TL	Total length
VCO_2	CO_2 production

Introduction

As an attempt to understand how animals respond, at different phenotypic levels, to diverse environmental scenarios in time and space, Weiner (1992) proposed the “barrel model”. In this model, an organism is represented by a barrel, with input energy constraints—*foraging, digestion and absorption*—symbolized by funnels connected in tandem, and energy outputs—*maintenance, growth, and reproduction*—symbolized by a series of spouts arranged in parallel. Accordingly, when environmental conditions change, organisms are able to respond by adjusting the size of the funnels, the fluid stored inside the barrel, or the output flow through the spouts.

Adjustments of digestive features may be considered the classical physiological “funnels” in which phenotypic

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flexibility has been analyzed (see Starck 1999; McWilliams and Karasov 2001; Naya and Bozinovic 2004; Naya et al. 2007a). This is not surprising given that the digestive tract represents the functional link between energy intake and the energy available to fulfill all the vital functions (Karasov 1990; Bozinovic 1993; Secor 2001). In addition, it comprises one of the most costly tissues to maintain (Cant et al. 1996), and thus, adjusting digestive tract size to functional demands could represent an important energy-saving mechanism (Dietz and Piersma 2007).

On the other hand, when environmental conditions change, animals can also modify the “amount of fluid stored inside the barrel”, that is, the amount of energy stored in their tissues. In vertebrates, the most widespread and usually the most important energy storage organ is the adipose tissue, which is metabolically specialized for lipid deposition (Pond 1978). Among lizards, lipids are mainly stored in the abdominal fat bodies and along the tail (Smith 1968; Pond 1978), and available data indicate that the amount of lipid in these organs is correlated with the organism’s overall lipid reserves (e.g., Derickson 1976; Loumbourdis 1987). In these animals, energy reserves have been reported to change with several factors, like food availability (e.g., Bustard 1967; Gist 1972; Naya and Bozinovic 2006), reproductive demands (e.g., Hahn and Tinkle 1965; Smith 1968; Telford 1970; Lin 1979), wet and dry seasons (e.g., Sexton et al. 1971), and hibernation (e.g., Dessauer 1955; Derickson 1974).

Finally, during periods of nutritional bottlenecks or enhanced energetic demands, organisms may regulate the energy expenditure processes. In this sense, depression of metabolic rate in response to different environmental demands has been recorded for virtually all major animal phyla (see Guppy and Withers 1999; Makarieva et al. 2006). In ectothermal vertebrates, the standard metabolic rate (SMR) has been chosen as a good indicator of the energetic cost of maintenance (Ashby 1998), and thus, analysis of this variable is essential for determining whether ectothermic animals modulate their maintenance costs in response to different environmental conditions (Chown and Gaston 1999). SMR has been reported to change in response to diverse factors, such as food quantity (e.g., Anderson 1993) and quality (e.g., Zanotto et al. 1997; Simandle et al. 2001), reproductive state (e.g., Angilletta and Sears 2000; Finkler and Cullum 2002; Finkler 2006), and seasonality (e.g., Abe 1995; Christian et al. 1999).

Animals inhabiting highly variable environments usually exhibit a large amount of physiological flexibility to cope with these changes. Accordingly, they represent an excellent model to advance our understanding of the interplay between processes of energy acquisition, storage, and expenditure. In the present study, we examined the seasonal variation in the size of several organs and SMR in a lizard species (*Liolaemus bellii* = *L. altissimus bellii*), which

inhabits high-altitude habitats in the Andes range. These environments are characterized by snowy winter months of low temperatures and warm and dry summers, a thermal seasonal change that is related to the variation in many other physical and biotic conditions (Bozinovic et al. 1990).

Materials and methods

Model species, study site and specimen collection

Liolaemus bellii is a middle-sized, viviparous and omnivorous lizard that inhabits high-altitude environments of central Chile (from 2,000 to 3,000 m above sea level) in the Andes range (Velo and Navarro 1988; Mella 2005). In our study site (Farellones, 33°30’S–70°25’W, 2,800 m above sea level), animals are highly active during spring and summer months (i.e., October to March), and this activity is greatly reduced from the middle of autumn (i.e., April and May). In this sense, laboratory data indicate that foraging activity stops at the end of March regardless of food availability (D.E. Naya unpublished data). During winter months (i.e., June to September) the site is covered by more than 2 m of snow and individuals are forced to hibernate in subnivean habitats; animals emerge from hibernation with the onset of the spring (i.e., late September to early October). Regarding the reproductive cycle, it is known that birth occurs during the late spring and early summer and that gestation period is about 60 days (Leyton and Valencia 1992). *L. bellii* produces between four and six offspring (in a single litter) per year, whose body mass at birth ranges from 0.6 to 0.9 g (D.E. Naya unpublished data).

Sixty-four adult individuals of *L. bellii* were hand collected throughout 1 year—early summer 1 (January 2006, $n = 15$), early autumn (April 2006, $n = 15$), late winter (September 2006, $n = 8$), middle spring (November 2006, $n = 15$), and early summer 2 (January 2007, $n = 11$)—in Farellones, central Chile. Specimens were transferred to the laboratory on the same day of capture, housed in individual plastic cages (15 × 30 × 20 cm), and kept in a room at ambient temperature and natural photoperiod. Body mass (m_b) of each individual was measured using an electronic balance (± 0.1 g; Sartorius GMBH, Germany), while snout-to-vent length (SVL) and total length (TL) were measured using a plastic ruler (± 1 mm). For each animal a body condition index was estimated as: BCI (g cm^{-3}) = $(m_b/\text{SVL}^3) \times (10^3)$ (see Laurie 1989).

Metabolic rate measurements

After capture, animals were fasted for 2 days and then their metabolic rate was assessed. CO_2 production ($V\text{CO}_2$) was measured in a computerized (Datacan V) open-flow

respirometry system (Sable Systems, NV, USA) previously calibrated with certified gases. VCO_2 was measured in a plexiglass metabolic chamber of 0.25 L, at ambient temperatures of 20 and 30°C. To avoid a potential bias due to a setup acclimation effect, half of the sample was first measured at 20°C and the other half was first measured at 30°C. The metabolic chamber received dried air at a rate of 200 ml min⁻¹ from mass flow controllers (Sierra Instruments, CA, USA), and the air was passed through CO₂ absorbent granules (Baralyme®) before entering the chamber. Lizards stay in the chamber for 5 min before measurements. CO₂ production was monitored two times per second during 1 h. Each record was automatically transformed and recorded in ExpeData software (Sable Systems). Standard metabolic rate was estimated as the mean value of the lowest 3-min samples recorded during the period of recording. Animal activity inside the metabolic chamber was determined directly by visual observation. Before and after each measurement, m_b was recorded in an electronic balance (± 0.1 g).

Morphological determination

The next morning after VCO_2 measurements, lizards were cooled by decreasing ambient temperature to 4°C, and then killed through decapitation. Animals were then dissected and internal organs—stomach, small intestine, large intestine, liver, kidneys, heart, lungs, abdominal fat bodies and gonads—were removed and washed with Ringer's solution. Small and large intestine were completely empty of material and their length was measured with a ruler (± 0.5 mm). Small intestine width was measured in three sections (proximal, medium, and distal) with a digital caliper (± 0.01 mm; Mitutoyo, IL, USA). Small intestine nominal area was calculated as mean intestinal width \times length $\times 2$. Internal organs were dried to constant mass (1 week) in an oven at 60°C and then weighed (± 0.0001 g; Chyo JK-180, Japan). Animals' carcasses were dried to constant mass (10 days) in an oven at 60°C and then weighed (± 0.0001 g). Since the tail is an energy storage organ in *L. bellii* (Naya et al. 2007b), we determined the tail energy density for five randomly chosen specimens in each collection period. This variable was assessed in a Parr 1261 computerized calorimeter (Parr Instruments, IL, USA) on a dry basis.

Statistical analyses

Differences between seasons in body size (i.e., m_b , SVL, TL), body condition and tail energy density were evaluated separately using one-way ANOVAs. Differences in all the remaining dependent variables were evaluated separately through one-way ANCOVAs. In these analyses we used the following variables as covariates: (1) SVL for linear mea-

asures of digestive organs and small intestine area, (2) carcass dry mass for organ dry mass, and (3) body mass for SMR. The effect of organ dry mass on SMR was evaluated using Pearson product–moment correlation coefficients. To remove the effect of body size on both variables we used the residuals of SMR with respect to body mass, and the residuals of each organ's mass with respect to carcass dry mass. The assumptions of normality and homogeneity of variance were examined using Kolmogorov–Smirnov and Levene tests, respectively. When necessary to meet assumptions, variables were log transformed (e.g., dry mass of several organs). Interactions between covariates and factors were checked using a parallelism test, and a separate slopes ANCOVA model was used when necessary (e.g., liver dry mass). Differences among sampling periods (post hoc comparisons) were evaluated by Tukey unequal-N HSD tests. Statistical significance was established at the 0.05 level. A significant interaction term between seasons and sex was observed only for gonad dry mass, and consequently, in this case analyses of covariance were conducted separately for each sex. All the analyses were performed using the statistical package STATISTICA® version 6.0 (StatSoft, OK, USA).

Results

We did not find differences in body size or body condition among seasons (Table 1). Small and large intestine lengths and large intestine dry mass showed larger values during summer and autumn; however, small intestine nominal area and small intestine dry mass—i.e., those variables more relevant from a digestive perspective—reached greater values during summer and spring (Fig. 1a, Table 2).

Regarding energy storage, liver and fat body mass decreased from summer to winter, and then did not change from winter to spring (except for the case of males' fat bodies which were further reduced); in both sexes, the size of these organs was greatly increased during the next summer (Fig. 1b, c). Tail energy density was higher during summer months, did not change between summer and autumn, and then continuously decreased from autumn to spring (Fig. 1d).

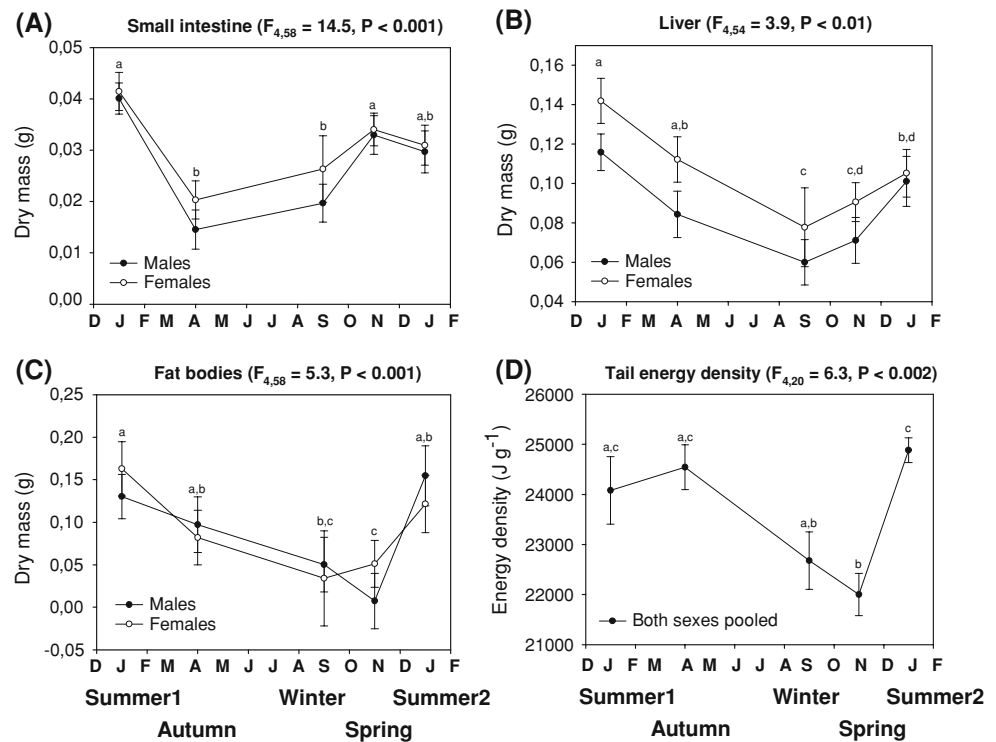
By contrast, the weight of gonads reached a maximum value during winter (males) or during winter and spring months (females), and then a conspicuous decrease during the following months was observed (Fig. 2). The largest mass of females' gonads in winter and spring was associated with the presence of ovulatory follicles or oviducal eggs (mean number = 6.1 ± 0.5), which were observed in all of the examined females ($n = 10$) during these seasons. Kidney dry mass also reached greater values during winter months, while lungs appear to follow an inverse trend (Table 2). Stomach and heart did not appear to follow a

Table 1 Body mass (m_b), snout-vent length (SVL), total length (TL), and body condition index (BCI) for each sampling period

Season	Summer 1 ($n = 15$)	Autumn ($n = 15$)	Winter ($n = 8$)	Spring ($n = 15$)	Summer 2 ($n = 11$)	F and P values
m_b (g)	12.1 (± 0.6)	11.1 (± 0.6)	10.5 (± 0.8)	12.1 (± 0.6)	10.3 (± 0.7)	$F_{4,59} = 1.59$ $P < 0.19$
SVL (cm)	7.3 (± 0.1)	7.1 (± 0.1)	7.1 (± 0.2)	7.3 (± 0.1)	7.0 (± 0.1)	$F_{4,59} = 1.04$ $P < 0.40$
TL (cm)	15.4 (± 0.8)	17.7 (± 0.8)	17.6 (± 1.0)	17.9 (± 0.8)	16.6 (± 0.9)	$F_{4,59} = 1.71$ $P < 0.16$
BCI (g cm^{-3})	31.5 (± 1.2)	30.3 (± 1.2)	28.9 (± 1.6)	30.8 (± 1.2)	30.1 (± 1.4)	$F_{4,59} = 0.50$ $P < 0.74$

Values presented are absolute means \pm 1 SE. n Sample size

Fig. 1 Seasonal changes in **a** small intestine dry mass, **b** liver dry mass, **c** fat bodies dry mass, and **d** tail energy density. Values are least square adjusted means \pm 1 SE for organ dry masses (covariate: carcass dry mass = 2.6 g) or absolute means \pm 1 SE for tail energy density. Different letters indicate significant differences between means



clear pattern of variation through the annual cycle (Table 2).

Standard metabolic rate at 30°C changed during the year, with the highest values observed during summer and spring, and the lowest during autumn and winter (Fig. 3a). However, this difference in SMR between high- and low-activity seasons was much lower when measured at 20°C, and additional data collected by us (see Fig. 3 footnote for a methodological clarification) indicate that it disappears completely at 10°C (Fig. 3b). Residuals of SMR did not correlate with residuals of organ dry mass either at 30 or at 20°C.

Discussion

Reversible changes in an organism's traits in response to changing environmental conditions are often hypothesized

to be a response that increases biological fitness (Scheiner 1993, 2002; Pigliucci 2001, 2005; Ghalambor et al. 2007). In what follows, we discuss the seasonal adjustments observed in *Liolaemus bellii* within this framework of phenotypic flexibility. We begin by analyzing changes in digestive and other central organs, then in energy-storage related organs, and finally with the standard metabolic rate.

Food processing organs

Changes in digestive organ features have been thoroughly investigated during the last century, and nowadays, phenotypic flexibility at this level is recognized as one of the most relevant physiological adjustments to environmental changing conditions (Piersma and Lindstrom 1997; Pennisi 2005; Naya et al. 2007a). Nevertheless, most of our knowledge on this topic has come from the analysis of adjustments in

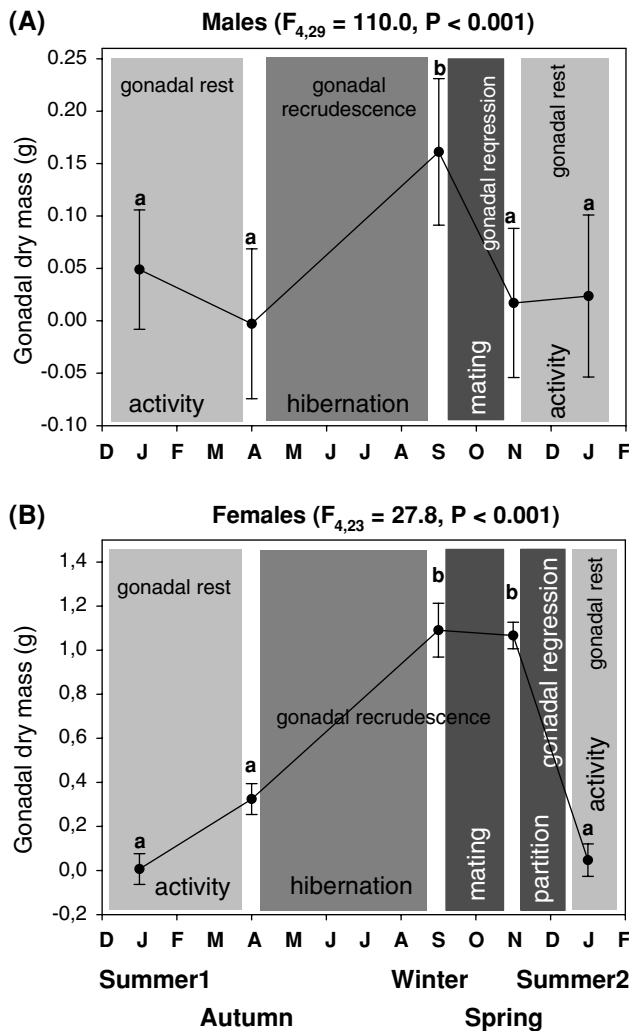


Fig. 2 Seasonal changes in gonad dry mass for **a** males, and **b** females. Values are least square adjusted means \pm 1 SE (covariate: carcass dry mass = 2.6 g). Different letters indicate significant differences between means. Gonadal activity periods (according to Leyton and Valencia 1992) are presented. Note gonads include the testes and epididymides in males and ovaries, oviducts and oviducal eggs (when present) in females

small endotherms (see Karasov and Diamond 1983; McWilliams and Karasov 2001; Dietz and Piersma 2007) or large ectotherms (see Secor et al. 1994; Secor and Diamond 2000; Starck et al. 2004; Ott and Secor 2007). In this sense, data from lizard species are very scarce, and, to our knowledge, are restricted to studies of seasonal changes in sugar and amino acid transport in the small intestine of *Uromastix hardwickii* (Latif et al. 1967; Qadri et al. 1970) and in brush-border uptake rates and gut morphology in *Sauromalous obesus* (Tracy and Diamond 2005), and to the effect of food availability in gut morphology of *Liolaemus nitidus* (Naya and Bozinovic 2006).

Here we found that *L. bellii* is able to adjust the size of its digestive organs through the annual cycle of activity. Specifically, we observed an increase in the small intestine

dry mass ranging between 50% (summer 2 vs. winter) and 100% (summer 1 vs. winter) from cold to warm months. After comparing our results with other small ectotherms, we observed that this value is similar to those recorded in hibernating and non-aestivating anuran and lizard species (Naya et al. 2005; Secor 2005; Tracy and Diamond 2005), but noticeably lower than those observed in aestivating anuran species (Cramp and Franklin 2003, 2005; Cramp et al. 2005; Secor 2005). In this sense, Bustard (1967) noted that in the case of fat stores, a greater amount of flexibility should be expected in species that aestivate than in species that hibernate. This is because in the former, inactivity occurs during warmer months, and thus, during a period of higher temperature-induced metabolic demand. Thus, it appears that Bustard’s idea on seasonal flexibility not only applies to energy storage organs, but also to organs with high-metabolic costs, such as digestive organs.

Other central organs

Heart mass did not change during the year, which is in agreement with what is known for *S. obesus* (Tracy and Diamond 2005) and also with small ectothermal species that aestivate (Secor 2005). The lack of regulation in this organ is probably related to the fact that cardiac performance must be maintained during the period of inactivity (Loveridge and Wither 1981; Secor 2005). On the other hand, kidney size reached the highest value during winter months, a result that is coincident with observations in other hibernating lizards, such as *S. obesus* (Tracy and Diamond 2005) and *Liolaemus nigroviridis* (D. E. Naya unpublished data), but for which we do not have a clear explanation.

Energy storage and reproductive organs

In contrast to digestive organs, there is a myriad of papers that analyze seasonal changes in the size of energy storage and reproductive organs. Indeed, given the early suggestion that ovarian hormones exert some regulatory influence on lipid mobilization (Hahn and Tinkle 1965; Smith 1968; Greenberg and Gist 1985), in most of the cases, changes in gonads and fat bodies have been analyzed simultaneously (e.g., Goldberg 1974; Jameson and Allison 1976; Lin 1979; Etheridge et al. 1986; Huang 1998). These studies indicated that, in temperate lizard species, lipid storage occurs largely during late summer or early autumn, and these reserves are mainly used during winter and spring for maintenance, gamete production and other reproductive activities (e.g., Guillette and Casas 1981; Selcer 1987; Mendez et al. 1988). Regarding the reproductive cycle, it is accepted that—with some exceptions, such as some Mexican (Ramirez et al. 1998) and South American lizard species

Table 2 Small and large intestinal length (in cm), small intestine nominal area (in cm²), and organ dry masses (in mg) for each sampling period

Season	Summer 1 (n = 15)	Autumn (n = 15)	Winter (n = 8)	Spring (n = 15)	Summer 2 (n = 11)	F and P values
<i>Lengths</i>						
Small intestine	8.1 (±0.4) ^a	7.7 (±0.4) ^a	5.4 (±0.5) ^b	6.7 (±0.4) ^{a,b}	7.2 (±0.4) ^{a,b}	F _{4,58} = 5.4 P < 0.001
Large intestine	2.0 (±0.1) ^a	2.0 (±0.1) ^a	1.2 (±0.2) ^b	1.4 (±0.1) ^{b,c}	2.0 (±0.1) ^{a,c}	F _{4,58} = 9.1 P < 0.001
<i>Small intestine nominal area</i>	4.3 (±0.3) ^a	2.2 (±0.3) ^b	1.7 (±0.4) ^b	3.6 (±0.3) ^a	3.4 (±0.4) ^a	F _{4,58} = 9.8 P < 0.001
<i>Dry masses</i>						
Stomach	36.0 (±1.8)	30.9 (±2.0)	29.7 (±2.5)	33.8 (±1.8)	36.7 (±2.2)	F _{4,58} = 1.9 P = 0.13
Large intestine	21.1 (±1.6) ^a	17.2 (±1.8) ^a	10.6 (±2.2) ^b	14.0 (±1.6) ^{b,c}	20.3 (±1.9) ^{a,c}	F _{4,58} = 5.3 P < 0.001
Kidneys	11.2 (±1.6) ^a	13.8 (±1.7) ^{a,b}	25.1 (±2.2) ^b	17.7 (±1.6) ^{a,b}	11.7 (±1.8) ^a	F _{4,58} = 2.8 P < 0.05
Heart	5.6 (±0.7)	5.7 (±0.8)	4.4 (±1.0)	5.9 (±0.7)	5.1 (±0.8)	F _{4,58} = 1.4 P = 0.23
Lungs	17.8 (±0.8) ^a	15.6 (±0.9) ^{a,b}	13.9 (±1.1) ^b	16.8 (±0.8) ^{a,b}	14.6 (±1.0) ^{a,b}	F _{4,58} = 2.9 P < 0.05

Values presented are least square adjusted means ± 1 SE—covariate for intestinal lengths and area: snout-to-vent length (overall mean value = 7.2 cm); covariate for organ dry masses: carcass dry mass (overall mean value: 2.6 g). Different letters denote significant difference between means. n Sample size

(Ramirez 1991) that exhibit reproduction in autumn—gonadal regression is complete before the dormancy period, recrudescence occurs during autumn and winter months, and the maximum development is reached before spring or summer (e.g., James and Shine 1985; Etheridge et al. 1986; Huang 1998).

The pattern of seasonal variation that we found for energy storage and gonads of *L. bellii* agrees, in general terms, with what is known for other temperate species. Specifically, the main changes observed can be summarized as follows:

1. From summer to autumn (January–April): fat bodies and liver size, which have reached a peak value during summer, decreased. This reduction probably is related to the use of lipid reserves for maintenance, since, as we mentioned above, foraging activity of *L. bellii* in the laboratory endogenously stops at the end of March. Moreover, the use of fat bodies in early autumn, before the beginning of hibernation and when food is still abundant has been previously reported in reptiles (see Gregory 1982). Gonads of both sexes are at rest during summer and early autumn.
2. From autumn to winter (April–September): the mass of fat bodies and liver, as well as the energy density of the tail, are reduced. This may be related to the costs of maintenance and gamete synthesis. In this sense, gonads of both sexes appear to begin recrudescence in late autumn, reaching a peak value during September. Gonads of females were, due to the presence of ovulatory follicles or oviducal eggs, an order of magnitude

greater than those of males. Given that the size of energy storage organs is reduced during winter in both sexes to a similar extent, and that gamete synthesis cost is much cheaper in males, it follows that (1) females are using energy reserves from other body sites (e.g., carcass), or (2) maintenance cost of males at this time is much higher than those of females. A previous study that analyzed changes in energy reserves—fat bodies, liver and the whole carcass—showed that males use fat three to four times faster during hibernation than do females (Jameson and Allison 1976).

3. From winter to spring (September–November): fat bodies and liver did not change in size, except for the case of males' fat bodies that are further reduced. We suspect that this decrease is related to males' reproductive activities (e.g., courtship, home range patrolling, and territorial defense), an association that has been observed in several other lizard species (e.g., Mendez et al. 1988; Castilla and Bauwens 1990). It should be noted that females' gonad size is practically the same in winter as in spring, which suggests that energy transfer into eggs is completed before the onset of spring.
4. From spring to summer (November–January): fat body, liver and tail energy density are greatly increased. Gonads of males remain at rest, while those of females undergo a period of regression. It should be noted that in the field we found some females near to parturition in January, but we did not work with these individuals. This means that in the present study all females collected at this time of the year were post-reproductive.

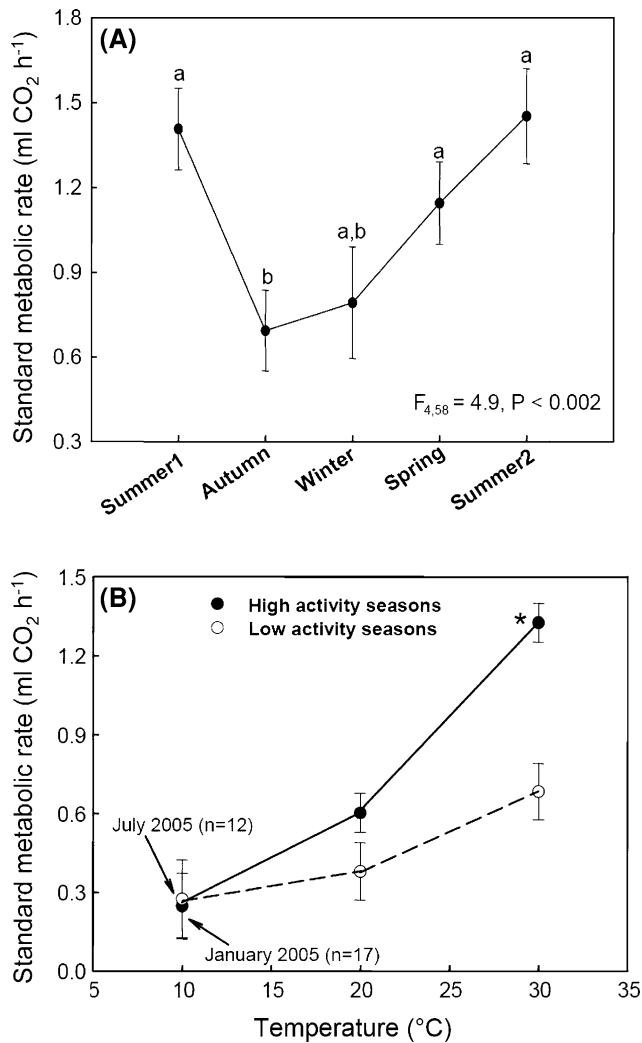


Fig. 3 **a** Standard metabolic rate (SMR) measured at 30°C for each season. **b** Pooled SMR for high activity seasons (spring and summers) and low activity seasons (autumn and winter) measured at 10, 20 and 30°C. Values are least square adjusted means \pm 1SE for organs' dry masses (covariate: body mass = 11.3 g). An asterisk indicates statistical differences with the other groups. Note: SMR measurements at 10°C comprise a small number of individuals recorded by the authors during the summer (January, $n = 17$) and winter (July, $n = 12$) of 2005. The methodology followed to obtain these data was the same as described in the main text

In the field, the post-reproductive state may be reached by some females later in the season (i.e., February).

Standard metabolic rate

The SMR of *L. bellii* measured at 30°C was noticeably greater during the high-activity seasons than during autumn and winter months. In this sense, a reduction in SMR during the low-activity periods has been previously recorded for other lizards that hibernate (Moberley 1963; Patterson and Davies 1978; Toledo et al. 2008) and also for species that aestivate (Abe 1995; see also Fig. 2 in Christian et al.

2003). Moreover, a quantitative analysis of our data indicates that metabolic depression during hibernation is about 50% of the maximum, a value that is in agreement with data for other reptiles (see Guppy and Whitters 1999). Interestingly, the seasonal pattern of variation in SMR was not evident at temperatures lower than 30°C, and differences in SMR between high- and low-activity periods were not observed at 20 or 10°C. This indicates that the temperature sensitivity (Q_{10}) of SMR is lower during cold seasons, a result that may be related to an organism's ability for physiological compensation at lower temperature (Seymour 1973; Lanciani et al. 1990; Burlando et al. 1992, but also see Clarke 1993; Pörtner et al. 2006).

With regard to the proximal causes of variation in SMR, it has been proposed that changes in body composition—mainly in the size of the organs with high metabolic rates, such as heart, kidney, gut and liver—are of chief importance in determining the magnitude of SMR (Kornazewski and Diamond 1995; Cant et al. 1996). In our case, changes in organ mass were poor predictors of the observed changes in SMR. Thus, it appears that other mechanisms, such as a change in the specific metabolic activity of body tissues (e.g., muscle, heart, kidney, liver), are responsible for the variation in SMR (see Vezina and Williams 2002). In this vein, it is known that specific activity of digestive and associated organs is greatly reduced during periods of inactivity in small hibernating ectotherms (e.g., Latif et al. 1967; Qadri et al. 1970; Pasanen and Koskela 1974; Tracy and Diamond 2005). In any event, if a seasonal change in tissue-specific activity holds for *L. bellii* a remaining question is: why this difference is expressed at 30°C, but not at 20 or 10°C? One potential explanation could be that the chemical composition of organs changes during the year (e.g., enzymes vs. anti-freeze compounds), and that this variation determines a change in metabolic rate only at higher temperatures. Indeed, an increase in SMR, and its thermal dependence, correlated with a rise in protein concentration during the warm season has been previously reported in ectotherms (Burlando et al. 1992).

Conclusion

Hibernation—i.e., the ability to reduce metabolic cost of maintenance beyond the expected depression from a physical–chemical temperature dependent reduction alone—comprises a large part of the life cycle of many temperate lizard species. This ability is related to the geographical distribution of these animals (Bartlett 1976) and, more generally, with the evolutionary success of small ectotherm vertebrates (Pinder et al. 1992). Here, we demonstrate that hibernation is correlated with a great physiological flexibility that allows animals to adjust energy acquisition, storage

and expenditure processes according to current environmental conditions.

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References

- Abe AS (1995) Estivation in South American amphibians and reptiles. *Braz J Med Biol Res* 28:241–247
- Anderson JF (1993) Respiratory energetics of two Florida harvestmen. *Comp Biochem Physiol* 105A:67–72
- Angilletta MJ, Sears MW (2000) The metabolic cost of reproduction in an oviparous lizard. *Funct Ecol* 14:39–45
- Ashby PD (1998) The effect of standard metabolic rate on egg production in the acridid grasshopper, *Xanthippus corallipes*. *Am Zool* 38:561–568
- Bartlett P (1976) Winter energy requirements of *Sceloporus occidentalis* in the Mojave desert. *Comp Biochem Physiol* 55A:179–181
- Bozinovic F (1993) Fisiología ecológica de la alimentación y digestión en vertebrados: modelos y teorías. *Rev Chil Hist Nat* 66:375–382
- Bozinovic F, Novoa FF, Veloso C (1990) Seasonal changes in energy expenditure and digestive tract of *Abrothrix andinus* in the Andes range. *Physiol Zool* 63:1216–1231
- Burlando B, Bavastrello G, Arillo A (1992) Seasonal changes in the metabolism of the calcareous sponge *Clathrina clathrus* (Schmidt). *Comp Biochem Physiol* 101A:341–344
- Bustard HR (1967) Gekkonid lizards adapt fat storage to desert environments. *Science* 158:1197–1198
- Cant JP, McBride BW, Croom WJ Jr (1996) The regulation of intestinal metabolism and its impact on whole animal energetics. *J Anim Sci* 74:2541–2553
- Castilla AM, Bauwens D (1990) Reproductive and fat body cycles of the lizard, *Lacerta lepida*, in Central Spain. *J Herpet* 24:261–266
- Chown SL, Gaston KJ (1999) Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biol Rev* 74:87–120
- Christian KA, Bedford GS, Schultz TJ (1999) Energetic consequences of metabolic depression in tropical and temperate-zone lizards. *Aust J Zool* 24:171–181
- Christian KA, Webb JK, Schultz TJ (2003) Energetics of bluetongue lizards (*Tiliqua scincoides*) in a seasonal tropical environment. *Oecologia* 136:515–523
- Clarke A (1993) Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Funct Ecol* 7:139–149
- Cramp RL, Franklin CE (2003) Is re-feeding efficiency compromised by prolonged starvation during aestivation in the green striped burrowing frog, *Cyclorana alboguttata*. *J Exp Zool* 300A:126–132
- Cramp RL, Franklin CE (2005) Arousal and re-feeding rapidly restores digestive tract morphology following aestivation in green-striped burrowing frogs. *Comp Biochem Physiol* 142A:451–460
- Cramp RL, Franklin CE, Meyer EA (2005) The impact of prolonged fasting during aestivation on the structure of the small intestine in the green-striped burrowing frog, *Cyclorana alboguttata*. *Acta Zool (Stockholm)* 86:13–24
- Derickson WK (1974) Lipid deposition and utilization in the Sagebrush lizard, *Sceloporus graciosus*: its significance for reproduction and maintenance. *Comp Biochem Physiol* 49A:267–272
- Derickson WK (1976) Lipid storage and utilization in reptiles. *Am Zool* 16:711–724
- Dessauer HC (1955) Seasonal changes in the gross organ composition of the lizard, *Anolis carolinensis*. *J Exp Zool* 128:1–12
- Dietz MW, Piersma T (2007) Red knots give up flight capacity and defended food processing capacity during winter starvation. *Funct Ecol* 21:899–904
- Etheridge K, Wit LC, Sellers JC, Trauth SE (1986) Seasonal changes in reproductive condition and energy stores in *Cnemidophorus sexlineatus*. *J Herpet* 20:554–559
- Finkler MS (2006) Effect of temperature, sex, and gravity on the metabolism of small-mouthed salamanders, *Ambystoma texanum*, during the reproductive season. *J Herpet* 40:103–106
- Finkler MS, Cullum KA (2002) Sex-related differences in metabolic rate and energy reserves in spring-breeding small-mouthed Salamanders (*Ambystoma texanum*). *Copeia* 2002:824–829
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407
- Gist DH (1972) The effect of starvation and refeeding on carbohydrate and lipid reserves of *Anolis carolinensis*. *Comp Biochem Physiol* 43A:771–780
- Goldberg SR (1974) Reproduction of the mountain and lowland population of lizard *Sceloporus occidentalis*. *Copeia* 1974:176–182
- Greenberg DS, Gist DH (1985) Fat bodies and reproduction in female *Anolis carolinensis*. *J Exp Zool* 233:277–283
- Gregory PT (1982) Reptilian hibernation. In: Gans C, Pough H (eds) *Biology of Reptilia*. Academic Press, London, pp 53–154
- Guillette LJ Jr, Casas G (1981) Seasonal variation in fat body weights of the Mexican high elevation lizard *Sceloporus grammicus microlepidotus*. *J Herpet* 15:366–371
- Guppy M, Withers P (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev* 74:1–40
- Hahn WE, Tinkle DW (1965) Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*. *J Exp Zool* 158:79–86
- Huang W-S (1998) Reproductive cycles of the grass lizard, *Takydromus hsuehshanensis*, with comments on reproductive patterns of lizards from the central high elevation area of Taiwan. *Copeia* 1998:866–873
- James CD, Shine R (1985) The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia* 67:464–474
- Jameson EW, Allison A (1976) Fat and breeding cycles in two montane populations of *Sceloporus occidentalis* (Reptilia, Lacertilia, Iguanidae). *J Herpet* 10:211–220
- Karasov WH (1990) Digestion in birds: chemical and physiological determinants and ecological implications. In: Morrison ML, Ralph CJ, Verner J, Jehl JR (eds) *Avian foraging: theory, methodology, and applications studies in avian biology* No. 13. Cooper Ornithological Society, Kansas, pp 391–415
- Karasov WH, Diamond JM (1983) Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *Am J Physiol* 245:G443–G462
- Konarzewski M, Diamond J (1995) Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* 49:1239–1248
- Lanciani C, Giesel JT, Anderson JF (1990) Seasonal change in metabolic rate of *Drosophila simulans*. *Comp Biochem Physiol* 97A:501–504
- Latif SA, Zain BK, Zain-ul-Abidin M (1967) Intestinal transport of sugar in a lizard during hibernation and activity. *Comp Biochem Physiol* 23:121–128
- Laurie WA (1989) Effects of the 1982–83 El Niño-Southern Oscillation event on marine iguana (*Amblyrhynchus cristatus*) populations in the Galapagos islands. In: Glynn P (ed) *Global ecological*

- consequences of the 1982–83 El Niño–Southern Oscillation. Elsevier, New York, pp 121–141
- Leyton V, Valencia J (1992) Follicular population dynamics: its relation to clutch and litter size in Chilean *Liolaemus* lizards. In: Hamlett W (ed) Reproductive biology of South American vertebrates. Springer, New York, pp 123–134
- Lin E (1979) Fatbody and liver cycles in two tropical lizards, *Chamaeleo hohneli* and *Chamaeleo jacksoni*. *J Herpetol* 13:113–117
- Loumbourdis NS (1987) Lipid storage and utilization in the lizard *Agama seillo seillo*. *J Herpetol* 21:237–239
- Loveridge JP, Wither PC (1981) Metabolism and water balance of active and cocooned African bullfrogs, *Ptychocheilichthys adspersus*. *Physiol Zool* 54:203–214
- Makarieva AM, Gorshkov VG, Li B-L, Chown SL (2006) Size- and temperature-independence of minimum life-supporting metabolic rates. *Funct Ecol* 20:83–96
- McWilliams SR, Karasov WH (2001) Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp Biochem Physiol* 128A:579–593
- Mella JE (2005) Guía de campo reptiles de Chile: zona central. Ediciones del Centro de Ecología Aplicada, Santiago de Chile
- Mendez FR, Guillelte LJ Jr, Villagrán Santa Cruz M, Casas-Andreu G (1988) Reproductive and fat body cycles of the viviparous lizard, *Sceloporus mucronatus* (Sauria: Iguanidae). *J Herpetol* 22:1–12
- Moberley WR (1963) Hibernation in the desert iguana, *Dipsosaurus dorsalis*. *Physiol Zool* 36:152–160
- Naya DE, Bozinovic F (2004) Digestive phenotypic flexibility in post-metamorphic amphibians: studies on a model organism. *Biol Res* 37:365–370
- Naya DE, Farfán G, Sabat P, Méndez MA, Bozinovic F (2005) Digestive morphology and enzyme activity in the Andean toad *Bufo spinulosus*: hard-wired or flexible physiology? *Comp Biochem Physiol* 140A:165–170
- Naya DE, Bozinovic F (2006) The role of ecological interactions on the physiological flexibility of lizards. *Funct Ecol* 20:601–608
- Naya DE, Karasov WH, Bozinovic F (2007a) Phenotypic plasticity in laboratory mice and rats: a meta-analysis of current ideas on gut size flexibility. *Evol Ecol Res* 9:1363–1374
- Naya DE, Veloso C, Muñoz JL, Bozinovic F (2007b) Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comp Biochem Physiol* 146A:189–193
- Ott BD, Secor SM (2007) Adaptive regulation of digestive performance in the genus Python. *J Exp Biol* 210:340–356
- Pasanen S, Koskela P (1974) Seasonal and age variation in the metabolism of the common frog, *Rana temporaria* L. in northern Finland. *Comp Biochem Physiol* 47A:635–654
- Patterson JW, Davies PMC (1978) Energy expenditure and metabolic adaptation during winter dormancy in the lizard *Lacerta vivipara* Jacquin. *J Therm Biol* 3:183–186
- Pennisi E (2005) The dynamic gut. *Science* 307:1896–1899
- Piersma T, Lindstrom A (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol Evol* 12:134–138
- Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. John Hopkins University Press, Baltimore
- Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol* 20:481–486
- Pinder A, Storesy K, Ultsch G (1992) Estivation and hibernation. In: Feder ME, Burggren WW (eds) Environmental physiology of the amphibians. University of Chicago Press, Chicago, pp 250–274
- Pond CM (1978) Morphological aspects and mechanical consequences of fat deposition in wild vertebrates. *Annu Rev Ecol Syst* 9:519–570
- Pörtner HO, Bennett AF, Bozinovic F, Clarke A, Lardies MA, Lenski RE, Lucassen M, Pelster B, Schiemer F, Stillman JH (2006) Trade-offs in thermal adaptation: in need of a molecular to ecological integration. *Physiol Biochem Zool* 79:295–313
- Qadri M-O-N, Zain B, Zain-ul-Abidin M (1970) Intestinal transport of amino acids in a lizard during hibernation and activity. *Comp Biochem Physiol* 36:569–577
- Ramirez MP (1991) Reproductive and fat body cycles of the viviparous lizard *Liolaemus huacahuasicus*. *J Herpetol* 25:205–208
- Ramirez A, Barba-Torres J, Vitt LJ (1998) Reproductive cycle and brood size of *Eumeces lynxe* from Pinal de Amoles, Queretero, México. *J Herpetol* 32:18–24
- Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol Syst* 24:35–68
- Scheiner SM (2002) Selection experiments and the study of phenotypic plasticity. *J Evol Biol* 15:889–898
- Selcer KW (1987) Seasonal variation in fatbody and liver mass of the introduced Mediterranean gecko, *Hemidactylus turcicus*, in Texas. *J Herpetol* 21:74–78
- Secor SM (2001) Regulation of digestive performance: a proposed adaptive response. *Comp Biochem Physiol* 128A:565–577
- Secor SM (2005) Physiology response to feeding, fasting and aestivation for anurans. *J Exp Biol* 208:2595–2608
- Secor SM, Diamond JM (2000) Evolution of regulatory responses to feeding in snakes. *Physiol Biochem Zool* 73:123–141
- Secor SM, Stein ED, Diamond J (1994) Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *Am J Physiol* 266:G695–G705
- Sexton EL, Ortleb EP, Hathaway LM, Ballinger RE, Licht P (1971) Reproductive cycles of three species of anoline lizards from the Isthmus of Panama. *Ecology* 52:201–215
- Seymour RS (1973) Energy metabolism of dormant spadefoot toads (*Scaphiopus*). *Copeia* 1973:435–445
- Simandle ET, Espinoza RE, Nussear KE, Tracy CR (2001) Lizards, lipids, and dietary links to animal function. *Physiol Biochem Zool* 74:625–640
- Smith RE (1968) Experimental evidence for a gonadal–fat body relationship in two teiid lizards (*Ameiva festiva*, *Ameiva quadrileata*). *Biol Bull* 134:325–331
- Starck JM (1999) Structural flexibility of the gastro-intestinal tract of vertebrates—implications for evolutionary morphology. *Zool Anz* 238:87–101
- Starck JM, Moser P, Werner RA, Linke P (2004) Pythons metabolize prey to fuel the response to feeding. *Proc Roy Soc Lond* 271B:903–908
- Telford SR (1970) Seasonal fluctuations in liver and fatbody weights of the Japanese lacertid *Takydromus tachydromoides* Schlegel. *Copeia* 1970:681–689
- Toledo LF, Brito SP, Milsom WK, Abe AS, Andrade DV (2008) Effects of season, temperature, and body mass on the standard metabolic rate of tegu lizards (*Tupinambis merianae*). *Physiol Biochem Zool* 81:158–164
- Tracy CR, Diamond J (2005) Regulation of gut function varies with life-history traits in chuckwallas (*Sauromalus obesus*: Iguanidae). *Physiol Biochem Zool* 78:469–481
- Veloso A, Navarro J (1988) Lista sistemática y distribución geográfica de anfibios y reptiles de Chile. *Boll Mus Reg Sci Nat (Torino)* 6:481–539
- Vezina F, Williams TD (2002) Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative capacity in breeding European Starlings: implications for metabolic rate and organ mass relationship. *Funct Ecol* 19:119–128
- Weiner J (1992) Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. *Trends Ecol Evol* 7:384–388
- Zanotto FP, Gouveia SM, Simpson SJ, Raubenheimer D, Calder PC (1997) Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *J Exp Biol* 200:2437–2448